

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number  
**WO 01/09169 A2**(51) International Patent Classification<sup>7</sup>: C07K 5/062, 5/065, C07D 295/12, A61K 38/05, 31/5375, 31/381, A61P 19/00, 31/00, 35/00, 37/00, 9/00

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(21) International Application Number: PCT/GB00/02830

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(22) International Filing Date: 21 July 2000 (21.07.2000)

(25) Filing Language: English

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(26) Publication Language: English

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(30) Priority Data:  
9917909.5 31 July 1999 (31.07.1999) GB

## Published:

— Without international search report and to be republished upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

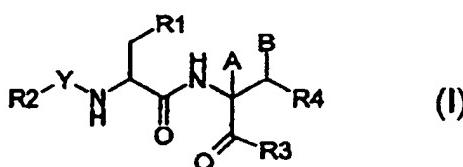
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(54) Title: CYSTEINE PROTEASE INHIBITORS



p-toluenesulfonic acid salts.

(57) Abstract: This invention relates to derivatives of alpha-amino acid amides, to pharmaceutical compositions containing such compounds, and to their use in medicine as inhibitors of cysteine proteases, particularly the cathepsins. A compound of formula (I) is described or a pharmaceutically acceptable salt, hydrate or solvate thereof. Pharmaceutically acceptable salts of the compounds of this invention include the sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and

### Cystein Protease Inhibitors

This invention relates to derivatives of alpha-amino acid amides, to pharmaceutical compositions containing such 5 compounds, and to their use in medicine as inhibitors of cysteine proteases, particularly the cathepsins.

#### Background to the Invention

The cathepsin family (C1) of lysosomal cysteine (or thiol) 10 proteases is a subset of the papain superfamily (clan CA of cysteine proteases) and includes cathepsin B, H, K, S, L and the recently discovered cathepsins O, O<sub>2</sub>/K, V, X, Z and W (lymphopain). Related enzymes also regarded as cysteine proteases are the cytoplasmic Ca<sup>2+</sup> dependent 15 calpains (family C2). Cysteine proteases are classified both functionally and according to the nature of their active site, which has a thiol residue. They differ in substrate specificities and other enzymatic activities, these differences probably arising from evolutionary 20 divergence.

The known cathepsins are synthesized on membrane bound 25 ribosomes, transferred to the endoplasmic reticulum, then to the Golgi apparatus and finally to the lysosome and endosomes. They have an important function in regulation of intracellular protein metabolism, mobilisation of tissue proteins and conversion of proenzymes, prohormones and neuropeptides into biologically active molecules. The 30 cathepsins are believed to be involved in a number of diseases.

Cathepsin K can be secreted into the extracellular space and is involved in bone and cartilage remodelling. Cath psin K is implicated in the pathogenesis of osteoporosis. Cathepsin K inhibitors can prevent 5 osteoporosis in animal models (PNAS. 1997. 94:14249-14254). Cathepsin L inhibitors have also been shown to inhibit osteoporosis (Bone, 1997. 20:465-471).

10 Cathepsin B and other cysteinyl cathepsins have also been shown to be released extracellularly by various tumour cells and are thought to play a role in tumour invasion (Journal of cellular Physiology. 1992. 150:534-544).

15 The cysteinyl cathepsins have also been shown to play a role in rheumatoid arthritis (Arthritis and Rheumatism 1994. 37:236-247) and neuronal and cardiac ischaemia (European Journal of Neuroscience. 1998. 10.1723-1733).

20 Cathepsins S and L both play a role in the generation of free MHC class II molecules capable of binding antigenic peptides in the endosomes. These class II/peptide complexes move to the cell membrane and are involved in T lymphocyte activation. Inhibitors of Cathepsin S have been shown to inhibit allergic immune responses (Journal of 25 Clinical Investigation. 1998. 101:2351-2363).

In addition to their role in the above diseases, cysteinyl cathepsins play a major role in the pathogenesis of 30 infectious diseases. For example, cysteinyl cathepsins are used by the protozoal parasites Plasmodium (malaria) and Trypanosoma (Chagas Disease) to invade the human host and

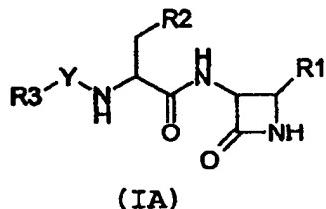
cysteinyl cathepsin inhibitors can inhibit experimental disease in both cases (Antimicrobial agents and chemotherapy. 1998. 42:2254-2258; Journal of Experimental Medicine. 1998. 188:725-734). Cysteinyl cathepsins are 5 also virulence factors for several pathogenic bacteria.

A recent review (Annu. Rev. Physiol. 1997. 59:63-88) describes the state of the art of cysteine proteases, including the cathepsins, and their presumed biological 10 functions. Other reviews deal with cathepsin B inhibitors as potential anti-metastatic agents (Exp. Opin. Ther. Patents, 1998, 8: 645-672), and cathepsin K inhibitors as potential treatments for osteoporosis (Exp. Opin. Ther. Patents, 1999, 9: 683-644).

15

International patent applications WO 96/32408, WO 98/12176, WO 98/12210 and GB 9805287.0 describe, inter alia, classes of cysteine protease inhibitors which may be represented by formula (IA):

20



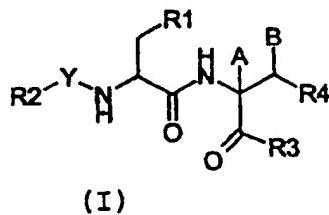
wherein Y, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> represent the groups found in corresponding positions of the compounds disclosed in 25 those publications. These known compounds are azetidin-2-ones which are monosubstituted at positions 3 and 4.

Brief Description of the Invention

The present invention makes available a new class of cysteine protease inhibitors which differ in structure from those disclosed in WO 96/32408, WO 98/12176, WO 5 98/12210 and GB 9806287.0 principally in that the azetidin-2-one ring is replaced by a substituted carbonylmethyl moiety, as more fully explained below. These compounds are useful for the treatment of diseases mediated by cysteine protease activity, for example 10 muscular dystrophy, osteoporosis, tumour metastasis, rheumatoid arthritis, neuronal or cardiac ischaemia, allergic immune response, and protozoal or bacterial disease.

15 Detailed Description of the Invention

According to the present invention, there is provided a compound of formula (I)



20

wherein:

Y represents -C(O)- or -S(O<sub>2</sub>)-;

25 R<sub>1</sub> represents a radical of formula R<sub>6</sub>-(ALK)<sub>p</sub>-(Z)<sub>n</sub>-(ALK)<sub>q</sub>- wherein Z represents -O- or -S-, ALK represents a divalent C<sub>1</sub>-C<sub>3</sub>alkyl or halogen-substituted C<sub>1</sub>-C<sub>3</sub>alkyl

radical, p and q are independently 0 or 1, n is 0 or 1 when q is 1 and n is 0 when q is 0, and R<sub>6</sub> is hydrogen or an optionally substituted C<sub>1</sub>-C<sub>6</sub>alkyl, C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-C<sub>6</sub>alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group; or R<sub>1</sub> together with the carbon atom to which it is attached forms a cycloalkyl ring;

R<sub>2</sub> represents -OR<sub>5</sub> or -R<sub>5</sub>;

R<sub>5</sub> represents a radical of formula R<sub>7</sub>-(A)<sub>t</sub>- wherein t is 0 or 1; A represents (i) an optionally substituted divalent C<sub>1</sub>-C<sub>6</sub>alkyl, radical which may be interrupted by one or more non-adjacent -O-, -S- or -NH- linkages, or (ii) a divalent C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-C<sub>6</sub>alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic radical, or (iii) a -NH- link; and R<sub>7</sub> represents hydrogen or an optionally substituted C<sub>1</sub>-C<sub>6</sub>alkyl, C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-C<sub>6</sub>alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group;

R<sub>3</sub> represents (i) an optionally substituted C<sub>1</sub>-C<sub>6</sub>alkyl, C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-C<sub>6</sub>alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group or (ii) NHR<sub>8</sub> or N(R<sub>8</sub>)<sub>2</sub> or (iii) OR<sub>8</sub> wherein R<sub>8</sub> represents hydrogen or an optionally substituted C<sub>1</sub>-C<sub>6</sub>alkyl, C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-C<sub>6</sub>alkynyl, cycloalkyl, cycloalkenyl or aryl;

A and B taken together represent a bond and R<sub>4</sub> represents a hydroxy or substituted hydroxy group or an amino or primary or secondary amino group, or A represents hydrogen and B and R<sub>4</sub> each independently represents a hydroxy or substituted hydroxy group;

or a pharmaceutically acceptable salt, hydrate or solvate thereof.

5    Pharmaceutically acceptable salts of the compounds of this invention include the sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid salts.

10   As used herein the term  $(C_1-C_6)$ alkyl or lower alkyl means a straight or branched chain alkyl moiety having from 1 to 6 carbon atoms, including for example, methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylprop-1-yl, 2-methylprop-2-yl, pentyl, 3-methylbutyl, 15 and hexyl. Similar terms such as " $(C_1-C_3)$ alkyl" are to be interpreted similarly.

As used herein the term  $C_2-C_6$ alkenyl" means a straight or branched chain alkenyl moiety having from 2 to 6 carbon atoms having at least one double bond of either E or Z stereochemistry where applicable. The term includes, for example, vinyl, allyl, 1- and 2-butenyl and 2-methyl-2-propenyl. Similar terms such as " $(C_2-C_3)$ alkenyl" are to be interpreted similarly.

25   As used herein the term " $C_2-C_6$  alkynyl" means a straight chain or branched chain hydrocarbon groups having from two to six carbon atoms and having in addition one triple bond. This term would include for example, ethynyl, 1-propynyl, 1- and 2-butynyl, 2-methyl-2-propynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 4-

hexynyl and 5-hexynyl. Similar terms such as "(C<sub>2</sub>-C<sub>3</sub>)alkynyl" are to be interpreted similarly.

As used herein the term cycloalkyl means a saturated  
5 alicyclic moiety having from 3-7 carbon atoms and  
includes, for example, cyclohexyl, cycloheptyl,  
cyclopentyl, cyclobutyl and cyclopropyl.

As used herein the term "halogen" means fluoro, chloro,  
10 bromo or iodo.

As used herein the term "aryl" refers to a mono-, bi- or  
tri-cyclic, substituted or unsubstituted, carbocyclic  
aromatic group, and to groups consisting of two covalently  
15 linked substituted or unsubstituted monocyclic carbocyclic  
aromatic groups. Illustrative of such groups are phenyl,  
biphenyl and napthyl. Examples include C<sub>6</sub>-C<sub>12</sub> aryl groups  
such as phenyl, biphenyl, naphthyl, tetrahydronaphthyl,  
dihydronaphthyl, and cyclohexyl phenyl.

20 As used herein the unqualified term heterocyclyl or  
heterocyclic means a 5-7 membered heterocyclic ring,  
which may be aromatic or non-aromatic, containing one or  
more heteroatoms selected from S, N and O, and optionally  
25 fused to a benzene or hetero-atom containing ring. The  
term therefore includes C<sub>1</sub>-C<sub>11</sub> heterocyclic groups  
containing 1-4 heteroatoms selected from nitrogen, sulfur  
or oxygen. Examples include thienyl, pyridyl, 1,2,3-  
triazolyl, 1,2,4-triazolyl, 1,2,3,4-tetrazolyl,  
30 imidazolyl, quinolinyl, isoquinolinyl, indolyl,  
pyrimidinyl, benzofuranyl, benzothienyl, morpholinyl,

- thiomorpholinyl, piperazinyl, piperidinyl,  
tetrahydroquinolinyl, tetrahydroisoquinolinyl,  
pyridylphenyl, pyrimidylphenyl, pyrrolyl, furyl, thieryl,  
piperidinyl, imidazolyl, oxazolyl, thiazolyl,  
5 thiadiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl,  
pyrimidinyl, morpholinyl, piperazinyl, indolyl,  
benzimidazolyl, maleimido, succinimido, and phthalimido  
groups.
- 10 As used herein, the term "primary or secondary amino group" means an amino group carrying one or two substituents respectively, for example selected from amino protecting groups,  $(C_1-C_6)$ alkyl-X-,  $(C_2-C_6)$ alkenyl-X-,  $(C_2-C_6)$ alkenyl-X-, aryl $(C_1-C_6)$ alkyl-X-, aryl $(C_2-C_6)$ alkenyl-X-,  
15 aryl $(C_2-C_6)$ alkenyl-X-, heterocyclic $(C_1-C_6)$ alkyl-X-, heterocyclic $(C_2-C_6)$ alkenyl-X-, heterocyclic $(C_2-C_6)$ alkenyl-X-, wherein -X- represents a bond or a carbonyl -C(O)-, sulphonyl -S(O<sub>2</sub>)-, or oxycarbonyl -O-C(O)- group, and wherein any of the foregoing may be substituted. The term  
20 "secondary amino group" also means a substituted or unsubstituted cyclic amino group, such as piperidyl, morpholinyl, thiomorpholinyl, piperazinyl, pyrrolidinyl or azetidinyl.
- 25 As used herein, the term "substituted hydroxy group" means a protected hydroxy group or a hydroxy group substituted by, for example, any of the substituents listed in the preceding paragraph as substituents of primary or secondary amino groups except those wherein X is an  
30 oxycarbonyl -O-C(O)- group.

As used herein in contexts other than "substituted hydroxy group", the unqualified term "substituted" as applied to a group or radical means substituted with 1, 2, or 3 substituents selected from

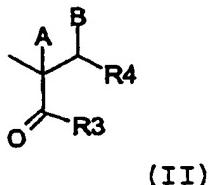
5

(C<sub>1</sub>-C<sub>3</sub>)alkyl;  
phenyl;  
hydroxy or mercapto;  
(C<sub>1</sub>-C<sub>3</sub>)alkoxy or (C<sub>1</sub>-C<sub>3</sub>)alkylthio;  
10 phenoxy or phenylthio;  
halogen;  
trifluoromethyl;  
nitro;  
cyano (-CN);  
15 carboxyl, and amidated, esterified or protected carboxyl;  
amino, mono- or di-(C<sub>1</sub>-C<sub>3</sub>)alkylamino, or protected amino;  
(C<sub>1</sub>-C<sub>3</sub>)alkylcarbonyl- or (C<sub>1</sub>-C<sub>3</sub>)alkylcarbonylamino-;  
20 -CONHR<sup>A</sup>, -NHR<sup>A</sup>, -NR<sup>A</sup>R<sup>B</sup>, or -CONR<sup>A</sup>R<sup>B</sup> wherein R<sup>A</sup> and R<sup>B</sup> are independently (C<sub>1</sub>-C<sub>3</sub>)alkyl; and  
-NH-C(=NR<sub>9</sub>)R<sub>10</sub> wherein R<sub>10</sub> is amino, mono- or di-(C<sub>1</sub>-C<sub>6</sub>)alkylamino, protected amino, or (C<sub>1</sub>-C<sub>3</sub>)alkyl, and R<sub>9</sub> is hydrogen, (C<sub>1</sub>-C<sub>3</sub>)alkyl, or an N-protecting group.  
25

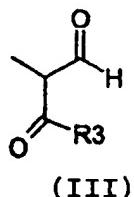
As used herein the term "protecting group" when used in relation to an amino, hydroxy or carboxylic acid moiety in the compounds of this invention means a group which is 30 used to render the amino, hydroxy or carboxylic acid moiety substantially non reactive, ie to neutralise its

amino, hydroxy or carboxylic acid functionality. In this context, protected amino groups include amido and acylamino, protected hydroxy groups include ethers, protected carboxyl groups include esters, and imidazolyl, 5 indolyl or guanidyl groups may be protected as t-butoxycarbonyl derivatives. These are only examples of the many protecting derivatives known in the art, and others will be known to the skilled man. Such protecting groups are of course well known, eg from the art of peptide synthesis, and are discussed in the widely used handbook by T.W. Greene and P.G.M. Wuts, Protective groups in Organic Synthesis, 2nd Edition, Wiley, New York 1991, and elsewhere in the chemical literature.

15 As mentioned above, the compounds of the invention differ in structure from those of WO 96/32408, WO 98/12176, WO 98/12210 and GB 9806287.0 principally in that the azetidin-2-one ring is replaced by a substituted carbonylmethyl moiety. That substituted carbonylmethyl 20 moiety is the radical (II):



which may be regarded as notionally derived from the aldehyde radical (III):



(III)

The substituents  $R_1$  and  $R_2$  in the compounds of the invention may be any of the groups falling within the 5 above definitions of  $R_1$  and  $R_2$  which are present in corresponding positions of cysteine protease inhibitors disclosed in WO 96/32408, WO 98/12176, WO 98/12210 and GB 9806287.0. Without prejudice to the generality of the foregoing, in the compounds of the invention:

10

$Y$  may be, for example,  $-C(O)-$ ;

$R_1$  may be, for example, a phenyl group which may be substituted by one or more of hydroxy, halogen, methoxy, 15 methyl, isopropyl, tert-butyl and trifluoromethyl; isopropyl, cyclohexyl; 3-pyridinyl; naphthyl; biphenyl; 2-thienyl; 3,4-methylenedioxyphenyl; 3,4-ethylenedioxy - phenyl; benzothienyl; thiazolyl; quinolinyl; isoquinolinyl; tetrahydroquinolinyl; tetrahydronaphthyl; 20 aminonaphthyl; or acetamidonaphthyl. Presently preferred are phenyl, isopropyl, cyclohexyl and 3-pyridinyl.

$R_2$  may be, for example, benzyloxy, 3-phenylpropyloxy, 3-phenylpropyl, 3-phenylprop-1-enyl, 6-N,N-25 dibenzyloxycarbonylguanidino-hexyl, 6-guanidino-hexyl, methoxy-methyleneoxy-methyl, 2-amino-ethoxy-methyl, 3-

(pyridin-3- or 4-yl)-propyl, or 3-(pyridin-3- or 4-yl)-prop-1-enyl.

R<sub>3</sub> may be, for example, methyl, ethyl, isopropyl, t-butyl,  
5 cyclohexyl, phenyl, 4-methoxyphenyl, 4-fluorophenyl,  
pyridyl, -NH<sub>2</sub>, methylamino, dimethylamino, benzylamino,  
piperidino, morpholino, piperazino, N-methylpiperazino or  
methoxy, ethoxy, t-butyloxy or phenoxy.

10 When A and B taken together represent a bond, R<sub>4</sub> may be,  
for example, -NH<sub>2</sub>, acetylamino, methylamino,  
dimethylamino, benzylamino, morpholino, piperidino,  
morpholino, piperazino or N-methylpiperazino,  
methoxycarbonylmethylamino, (methoxycarbonyl)-  
15 phenethylamino, -OH, methoxy, allyloxy, benzyloxy.

Specific compounds of the invention include those named  
and characterised in the Examples herein.

- 20 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-  
acetamido)-3-amino-acrylamide  
2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-  
acetamido)-3-amino-acrylamide  
2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3-  
25 amino-acrylamide  
2-[2S-2-(3-phenylpropionoyl)amino-2-benzyl-acetamido]-3-  
amino-acrylamide  
2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-  
acetamido)-3-benzylamino-acrylamide  
30 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-  
acetamido)-3-(morpholin-4-yl)-acrylamide

- 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(2-hydroxyethylamino)-acrylamide
- 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-phenylamino-acrylamide
- 5 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-piperidino-acrylamide
- 2E)-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-acetamido-acrylamide
- (2Z)-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-acetamido-acrylamide
- 10 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-acetamido-acrylamide
- 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3,3-dihydroxy-propionamide
- 2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3,3-dihydroxy-propionamide
- 15 2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3,3-dihydroxy-propionamide
- 2-[2S-2-(3-phenylpropionoylamino)-2-benzyl-acetamido]-3,3-dihydroxy-propionamide
- 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-hydroxy-acrylamide
- 20 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-benzylamino-N-benzyl-acrylamide
- 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(4-methylpiperazino)-acrylamide
- 25 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(3-tert-butoxycarbonylamino-pyrrolidino)-acrylamide
- (2E)-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-acetamido-acrylamide
- 30 (2Z)-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-acetamido-acrylamide

- tert-Butyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(4-methylpiperazino-1-methylenyl)-acetate
- Ethyl-2-[2S-(benzyloxycarbonylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- Ethyl-2-[2S-(benzyloxycarbonylamino)-2-cyclohexylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- Diphenylmethyl-2-[2S-(3-phenylpropionylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate
- 2-[2S-(Benzothiophen-2-carbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-4'-methoxyacetophenone.
- 2-[2S-(Benzylloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetophenone.
- 2-[2S-(Benzylloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-4'-methoxyacetophenone.
- 2-[2S-(Benzylloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-4'-fluoroacetophenone.
- (2E)-(2S-2-benzylloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamide
- (2Z)-(2S-2-benzylloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamide
- (2E)-(2S-2-benzylloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-methylamino)-acrylamide

(2E) - (2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido) -3-(1-carbomethoxy-methylamino)-acrylamide

As stated, the compounds of the invention are inhibitors  
5 of cysteine proteases, for example cathepsins B, L, S  
and/or K. The invention therefore also provides a  
pharmaceutical composition containing a compound of  
formula (I) as defined above, and a pharmaceutically  
acceptable carrier. Also provided is the use of such a  
10 compound in the preparation of a composition for  
inhibiting cysteine protease activity in the body of a  
mammal suffering a disease mediated by such activity, and  
a method of treatment of an animal suffering from a  
disease mediated by cysteine protease activity, which  
15 method comprises administering to the mammal a sufficient  
amount of a compound of formula (I) as defined above to  
inhibit such activity.

Diseases mediated by cysteine protease activity include  
20 muscular dystrophy, osteoporosis, tumour metastasis,  
rheumatoid arthritis, neuronal or cardiac ischaemia,  
allergic immune response, and protozoal or bacterial  
disease.

25 Compositions with which the invention is concerned may be  
prepared for administration by any route consistent with  
the pharmacokinetic properties of the active  
ingredient(s).

30 Orally administrable compositions may be in the form of  
tablets, capsules, powders, granules, lozenges, liquid or

gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as 5 binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or 10 silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous 15 or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, 20 syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as 25 glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

30 For topical application to the skin, the active ingredient(s) may be made up into a cream, lotion or

ointment. Cream or ointment formulations, which may be used for the drug, are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceutics such as the British

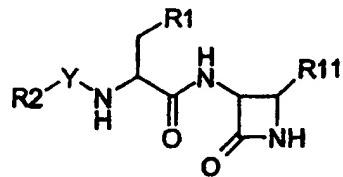
5 Pharmacopoeia.

The active ingredient(s) may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. Intravenous infusion is another route of administration for the compounds.

15

Safe and effective dosages for different classes of patient and for different disease states will be determined by clinical trial as is required in the art. It will be understood that the specific dose level for any 20 particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the 25 particular disease undergoing therapy.

Compounds of the invention wherein A and B taken together represent a bond and R<sub>4</sub> represents NH<sub>2</sub> may be prepared by treatment of azetidin-2-ones of formula (IV) with ammonium 30 hydroxide.



wherein R<sub>11</sub> is a leaving group such as phenoxy, acetoxy .

5

Compounds of the invention wherein A and B taken together represent a bond and R<sub>4</sub> represents a primary or secondary amino group may be prepared by treatment of compounds (IV) with a primary or secondary amine, or by appropriate 10 derivatisation of the amino group of the corresponding compounds wherein R<sub>4</sub> is amino.

Compounds of the invention wherein A and B taken together represent a bond and R<sub>4</sub> represents a hydroxy group may be 15 prepared by treatment of compounds (IV) with acetic acid, for example at ambient temperatures. Compounds of the invention wherein A represents hydrogen and B and R<sub>4</sub> represents a hydroxy group may also be prepared by treatment of compounds (IV) with acetic acid, but under 20 less forcing conditions than for the alpha-beta unsaturated compounds, for example at low temperatures such as about 0°C.

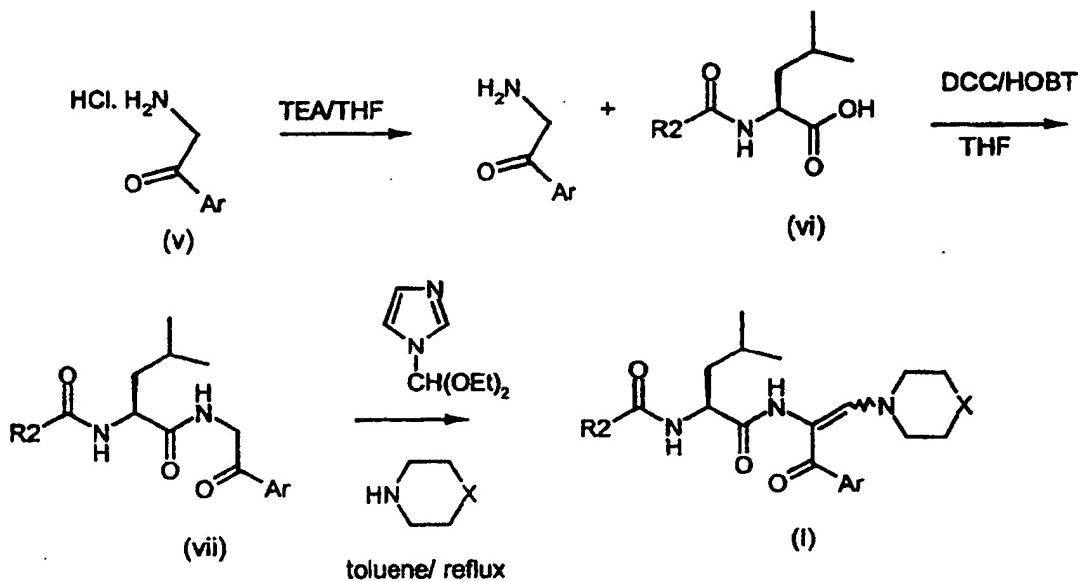
Compounds of the invention wherein A and B taken together 25 represent a bond and R<sub>4</sub> represents a substituted hydroxy group or a primary or secondary amino group may be prepared from the corresponding compounds wherein R<sub>4</sub> is

hydroxy or amino by appropriate derivatisation of that hydroxy or amino group. Likewise, compounds of the invention wherein A represents hydrogen and B and R<sub>4</sub> are independently a substituted hydroxy group may be prepared 5 from the corresponding compounds wherein B and R<sub>4</sub> are hydroxy by appropriate derivatisation of one or both of those hydroxy groups.

Compounds of the invention wherein A and B taken together 10 represent a bond and R<sub>4</sub> represents an alkyl, alkenyl, alkynyl, cycloalkyl or aryl may be prepared by the following the synthetic scheme as depicted below in scheme 1.

15

Scheme-1

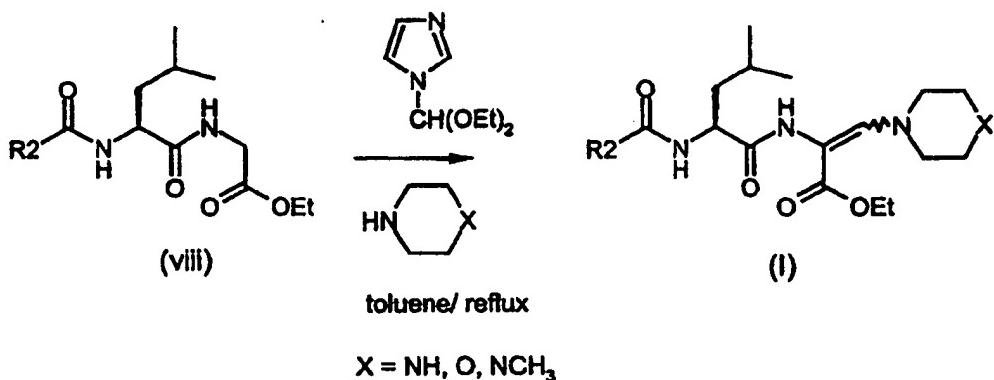


Compounds of the invention wherein A and B taken together represent a bond and R<sub>4</sub> represents an alkoxy, aryloxy or

cycloalkoxy may be prepared by the following the synthetic scheme as depicted below in scheme 2.

5

Scheme-2



In the above processes, the reactants are reacted together with solvent at elevated or low temperatures for sufficient time to allow the reaction to proceed to completion. The reaction conditions will depend upon the nature and reactivity of the reactants. Depending on the reactants, a solvent will generally be selected from the group consisting of benzene, toluene, acetonitrile, tetrahydrofuran, ethanol, methanol, chloroform, ethyl acetate, methylene chloride, dimethyl formamide, dimethyl sulfoxide, hexamethyl phosphoric triamide, water, pyridine, acetone and the like. Solvent mixtures may also be utilized.

Reaction temperatures generally range from between -70 °C to 150 °C. The preferred molar ratios of reactants are 1:1 to 5. The reaction time range from 0.5 to 72 hours, depending on the reactants.

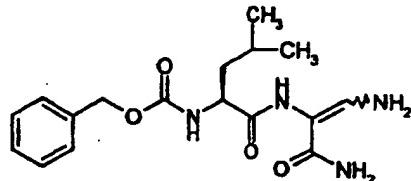
5

The azetidine-2-one strating materials (V) may be prepared by literature methods, including those in International patent applications WO 96/32408, WO 98/12176, WO 98/12210.

10 The following Examples illustrate embodiments of the invention.

Example 1

20 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-  
15 acetamido)-3-amino-acrylamide



A solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (3.0 g, 7.6 mmole) in acetonitrile 20 (50 ml) and 15 ml of ammonium hydroxide (28% NH<sub>3</sub> in water) was stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 1.86 g of the title 25 compound was obtained as white solid.

Yield: 70%

m.p.: 80-90 °C.

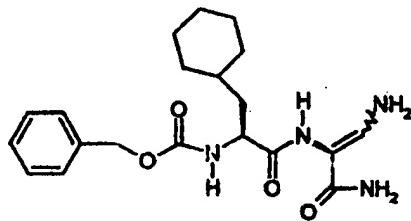
<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.7-1.0 (6H, m), 1.4-1.75 (3H, m), 3.9-4.1 (1H, m), 5.02 (2H, s), 5.56 (1.4H, br), 6.26 (2H, s), 6.44 (0.3H, t, J=9 Hz), 6.95 (0.6H, br), 7.12 (0.7H, t, J=9 Hz), 7.25-7.45 (5H, m), 7.36 (0.3H, d, J=6.6 Hz), 7.62 (0.7H, d, J=6.6 Hz), 8.50 (0.7H, s), 8.67 (0.3H, s).

MS (ES+): 349 (M+H), calcd for C<sub>17</sub>H<sub>24</sub>N<sub>4</sub>O<sub>4</sub> 348.

10

### Example 2

2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide



15

By a similar method as described in example 1, the title compound was obtained from (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-4-acetoxy-azetidin-2-one.

20 Yield: 70 %

m.p.: 80-85 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.7-1.95 (13H, m), 3.9-4.15 (1H, m), 5.01 (2H, s), 5.45-5.65 (1.6H, br), 6.26 (2H, s), 6.43 (0.2H, t, J=10 Hz), 6.9-7.0 (0.4H, br), 7.12 (0.8H, t, J=10 Hz), 7.3-7.45 (5H, m), 7.58 (0.2H, d,

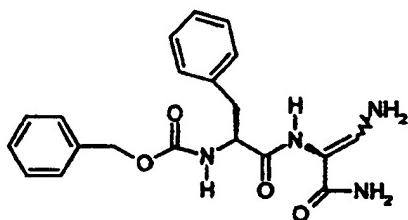
$J=6.4$  Hz), 7.62 (0.8H, d,  $J=6.4$  Hz), 8.51 (0.8H, s), 8.66 (0.2H, s).

MS (ES+): 389 (M+H), calcd for  $C_{20}H_{28}N_4O_4$  388.

5

**Example 3**

2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3-amino-acrylamide



10 By a similar method as described in example 1, the title compound was obtained from (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-4-acetoxyazetidin-2-one.

Yield: 71 %

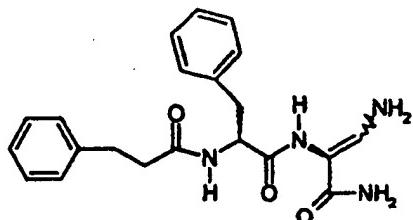
15 m.p.: 127-135 °C.

$^1H$ -NMR: (DMSO-d<sub>6</sub>), (ppm): 2.8-3.2 (2H, m), 4.2-4.4 (1H, m), 4.97 (2H, s), 5.3-5.55 (2H, br), 6.10 (2H, s), 7.14 (1H, t,  $J=11$  Hz), 7.2-7.4 (10H, m), 7.78 (1H, d,  $J=6.4$  Hz), 8.6 (1H, s).

20 MS (ES+): 383 (M+H), calcd for  $C_{20}H_{22}N_4O_4$  382.

## Example 4

2-[2S-2-(3-phenylpropionoyl)amino-2-benzyl-acetamido]-3-amino-acrylamide



5 By a similar method as described in example 1, the title compound was obtained from (3S, 4S)-3-[2S-2-(3-phenylpropionoyl)amino-2-benzyl-acetamido]-4-acetoxyazetidin-2-one.

Yield: 75 %

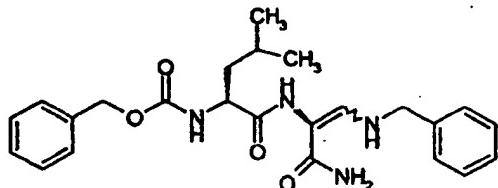
10 m.p.: 75-80 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 2.35-2.45 (2H, m), 2.70-3.15 (4H, m), 4.25-4.50 (1H, m), 5.25-5.50 (2H, br), 6.08 (2H, s), 7.06 (1H, t, J=11 Hz), 7.2-7.4 (10H, m), 8.32 (1H, d, J=6 Hz), 8.45 (1H, s).

15 MS (ES+): 381 (M+H), calcd for C<sub>21</sub>H<sub>24</sub>N<sub>4</sub>O<sub>3</sub> 380.

## Example 5

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethylacetamido)-3-benzylamino-acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (200 mg, 0.51 mmole) in acetonitrile (5 ml) and water (1 ml), benzylamine (542 mg, 5.1 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 200 mg of the title compound was obtained as white solid.

10 Yield: 90%

m.p.: 115-120 °C.

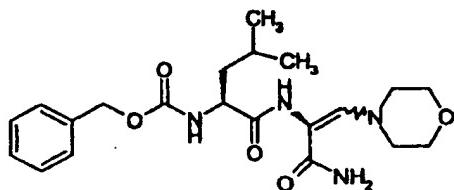
<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.8 (9H, m), 3.9-4.1 (1H, m), 4.29 (2H, d, J=5.5 Hz), 4.9-5.1 (2H, m), 5.9-6.1 (0.7H, m), 6.28 (2H, s), 6.61 (0.3H, d, J=12 Hz), 7.17 (0.7H, d, J=12 Hz), 7.25-7.45 (10H, m), 7.60 (0.3H, d, J=6.5 Hz), 7.66 (0.7H, d, J=6.5 Hz), 8.35-8.55 (0.3H, m), 8.60 (0.7H, s), 8.72 (0.3H, s).

MS (ES+): 439 (M+H), calcd for C<sub>24</sub>H<sub>30</sub>N<sub>4</sub>O<sub>4</sub> 438.

20

#### Example 6

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-(morpholin-4-yl)-acrylamide



25 To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (200 mg, 0.51 mmole) in

acetonitrile (5 ml) and water (1 ml), morpholine (444 mg, 5.1 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the solid was washed with ether. 200 mg  
5 of the title compound was obtained as white solid.

**Yield:** 90%

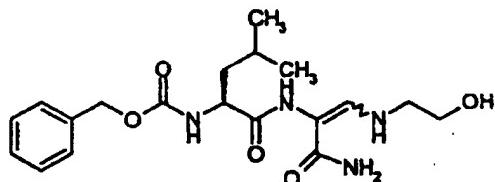
m.p.: 120-130 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-0.95 (6H, m), 1.3-1.75 (3H, m), 2.6-2.75 (4H, m), 3.5-3.6 (4H, m), 3.9-4.1 (1H, m), 5.00 (2H, s), 6.32 (2H, s), 7.07 (1H, s), 7.36 (5H, m), 7.67 (1H, d, J=6.7 Hz), 8.8 (1H, s).  
10 MS (ES+): 419 (M+H), calcd for C<sub>21</sub>H<sub>30</sub>N<sub>4</sub>O<sub>5</sub> 418.

15

#### Example 7

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-  
acetamido)-3-(2-hydroxyethylamino)-acrylamide



20 To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (5 ml) and water (1 ml), hydroethylamine (32 mg, 0.53 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel  
25

column chromatography using methanol-chloroform as eluant.  
70 mg of the title compound was obtained as white solid.

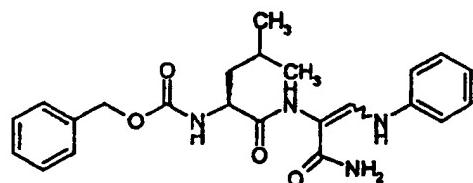
Yield: 70%

m.p.: 89-92 °C.

- 5  $^1\text{H-NMR}$ : (DMSO-d<sub>6</sub>), (ppm): 0.8-0.95 (6H, m), 1.4-1.75 (3H, m), 3.05-3.2 (2H, m), 3.35-3.5 (2H, m), 3.95-4.1 (1H, m), 4.65 (1H, t, J=5 Hz), 5.02 (2H, s), 5.4-5.55 (1H, m), 6.75 (2H, s), 7.12 (1H, d, J=12 Hz), 7.35 (5H, m), 7.65 (1H, d, J=6 Hz), 8.55 (1H, s).
- 10 MS (ES+): 393 (M+H), calcd for C<sub>19</sub>H<sub>28</sub>N<sub>4</sub>O<sub>5</sub> 392.

#### Example 8

- 15 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-phenylamino-acrylamide



Aniline hydrochloride (500 mg, 3.8 mmole) was neutralised with Na<sub>2</sub>CO<sub>3</sub> solution (600 mg, 5.7 mmole) and  
20 then extracted with ethyl acetate. After removal of solvent, aniline was dissolved in acetonitrile and added to a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (5 ml) and water (1 ml).  
25 The resulting mixture was stirred at room temperature overnight. After removal of solvent under vacuum and

lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 10 mg of the title compound was obtained as white solid.

**Yield:** 10%

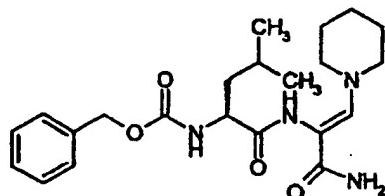
5 **m.p.:** 199-200 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.85-1.05 (6H, m), 1.5-1.8 (3H, m), 4.0-4.15 (1H, m), 5.09 (2H, s), 6.73 (2H, s), 6.88 (1H, t, J=7.2 Hz), 7.05 (2H, d, J=8 Hz), 7.24 (2H, d, J=7.5 Hz), 7.35 (5H, m), 7.7-7.9 (3H, m), 8.89 (1H, s).

10 **MS (ES+):** 425 (M+H), calcd for C<sub>23</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub> 424.

#### Example 9

15 **2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-piperidino-acrylamide**



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (3 ml) and water (1 ml), piperidine (88 mg, 1.06 mmole) was added and stirred at room temperature overnight. After removal of solvent under vacuum and lyophilization, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 70 mg of the title compound was obtained as white solid.

Yield: 70%

m.p.: 99-103 °C.

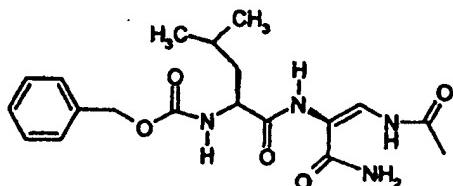
<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.7 (15H, m), 3.2-3.3 (4H, m), 3.95-4.1 (1H, m), 5.01 (2H, s), 6.23 (2H, s), 7.08 (1H, s), 7.3-7.4 (5H, m), 7.63 (1H, d, J=6 Hz), 8.76 (1H, s).

MS (ES+): 417 (M+H), calcd for C<sub>22</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub> 416.

10

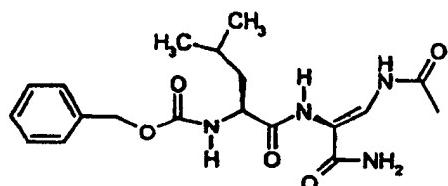
### Example 10a and 10b

(2E) - (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido) -3-acetamido-acrylamide (10a)



15

(2Z) - (2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido) -3-acetamido-acrylamide (10b)



20

150 mg (0.43 mmole) of 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido) -3-amine-acrylamide (from example 1) was dissolved in acetic anhydride (5 ml) and stirred at room temperature for 2 days. After removal of acetic anhydride, the residue was

purified by silica gel column chromatography using methanol-chloroform as eluant. 40 mg of the title compound (10a) and 45 mg of the title compound (10b) were obtained as white solid.

5 For (10a):

Yield: 24 %

m.p.: 73-76 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.0 (6H, m), 1.45-1.75 (3H, m), 2.08 (3H, s), 3.95-4.1 (1H, m), 5.02 (2H, s), 6.90 (1H, br), 7.08 (1H, d, J=12 Hz), 7.3-7.4 (5H, m), 7.5 (1H, br), 7.70 (1H, d, J=6 Hz), 9.23 (1H, s), 10.98 (1H, d, J=12 Hz).

MS (ES+): 391 (M+H), calcd for C<sub>19</sub>H<sub>26</sub>N<sub>4</sub>O<sub>5</sub> 390.

For (10b):

15 Yield: 27 %

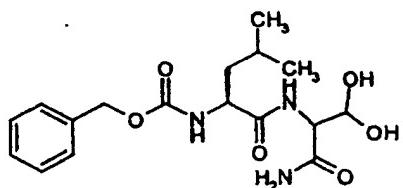
m.p.: 120-123 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.0 (6H, m), 1.5-1.8 (3H, m), 2.01 (3H, s), 4.0-4.1 (1H, m), 5.06 (2H, s), 7.02 (2H, s), 7.3-7.45 (5H, m), 7.64 (1H, d, J=11.4 Hz), 7.84 (1H, d, J=6.2 Hz), 9.00 (1H, s), 9.13 (1H, d, J=11.4 Hz),

MS (ES+): 391 (M+H), calcd for C<sub>19</sub>H<sub>26</sub>N<sub>4</sub>O<sub>5</sub> 390.

Example 11

25 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-  
acetamido)-3,3-dihydroxy-propionamide



To a solution of 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amine-acrylamide (30 mg, 0.086 mmole) (from example 1) in acetonitrile (2 ml) and water (0.5 ml), 5 drops of formic acid was added at 0 oC.

5 The mixture was stirred at 0 oC for 1 hr. After removal of acetonitrile under vacuum, precipitate was formed by addition of water (2 ml). The solid was purified by silica gel column chromatography using methanol-chloroform as eluant. 10 mg of the title compound was obtained as white solid.

**Yield:** 32%

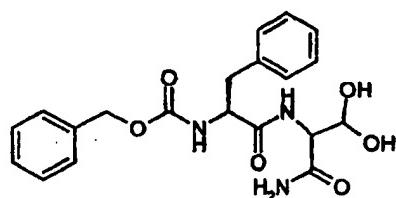
m.p.: 86-90 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.0 (6H, m), 1.4-1.75  
 (3H, m), 4.0-4.3 (2H, m), 4.6-4.8 (1H, m), 5.02 (1H,  
 15 s), 5.03 (1H, s), 6.35-6.45 (1H, m), 6.6-6.7 (1H, m),  
 7.2 (2H, br), 7.3-7.4 (5H, m), 7.5-7.9 (2H, m).

MS (ES+): 350 (M-H<sub>2</sub>O+H), calcd for C<sub>17</sub>H<sub>25</sub>N<sub>3</sub>O<sub>6</sub> 367.

### Example 12

20 2-(2S-2-benzylloxycarbonylamino-2-cyclohexylmethyl-  
acetamido)-3,3-dihydroxy-propionamide



By a similar method as described in example 11, the title compound was obtained from 2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide (from example 2).

Yield: 45%

m.p.: 105-110 °C.

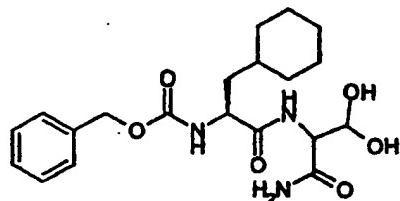
<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.8 (13H, m), 4.0-4.3 (2H, m), 4.6-4.85 (1H, m), 5.03 (2H, m), 6.3-6.7 (2H,

5 m), 7.14 (2H, br), 7.3-7.4 (5H, m), 7.45-7.9 (2H, m).

MS (ES+): 390 (M-H<sub>2</sub>O+H), calcd for C<sub>20</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub> 407.

### Example 13

2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3,3-  
10 dihydroxy-propionamide



By a similar method as described in example 11, the title compound was obtained from 2-(2S-2-benzyloxycarbonylamino-2-benzyl-acetamido)-3-aminoacrylamide (from example 3).

Yield: 40%

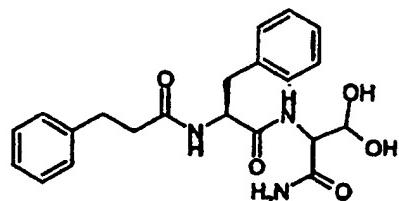
m.p.: 98-103 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 2.7-3.15 (2H, m), 4.2-4.5 (2H, m), 4.65-4.8 (1H, m), 4.94 (2H, s), 6.35-6.5 (1H, m), 6.6-6.75 (1H, m), 7.1-7.45 (12H, m), 7.5-7.65 (1H, m), 7.9-8.15 (1H, m).

MS (ES+): 384 (M-H<sub>2</sub>O+H), calcd for C<sub>20</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub> 401.

## Example 14

2-[2S-2-(3-phenylpropionoylamino)-2-benzyl-acetamido]-3,3-dihydroxy-propionamide



5

By a similar method as described in example 11, the title compound was obtained from 2-(2S-2-(3-phenylpropionoylamino)-2-benzyl-acetamido)-3-aminoacrylamide (from example 4).

10 Yield: 48%

m.p.: 105-110 °C.

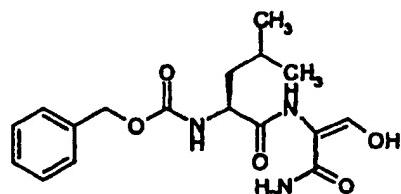
<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 2.3-2.4 (2H, m), 2.6-3.2 (4H, m), 4.2-4.3 (1H, m), 4.5-4.8 (2H, m), 6.3-6.45 (1H, m), 6.55-6.7 (1H, m), 7.1-7.45 (12H, m), 7.85-8.35 (2H, m).

15 MS (ES+): 382 (M-H<sub>2</sub>O+H), calcd for C<sub>21</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub> 399.

20

25

## Example 15

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-hydroxy-acrylamide

5

To a solution of 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-amine-acrylamide (55 mg, 0.158 mmole) (from example 1) in acetonitrile (3 ml) and water (0.5 ml), 10 drops of formic acid was added at 0 oC.

10 The mixture was stirred at room temperature for 1 hr. After removal of solvent under vacuum, the residue was purified by silica gel column chromatography using methanol-chloroform as eluant. 20 mg of the title compound was obtained as white solid.

15 Yield: 36%

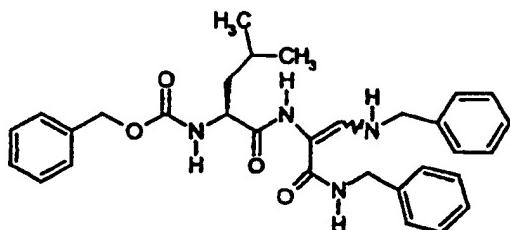
m.p.: 105-115 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.0 (6H, m), 1.4-1.8 (3H, m), 3.9-4.15 (1H, m), 5.02 (2H, s), 6.5-7.1 (1.5H, m), 7.3-7.5 (7H, m), 7.65-7.8 (1H, m), 9.05 (1H, s),  
20 10.2 (0.5H, m).

MS (ES+): 350 (M+H), calcd for C<sub>17</sub>H<sub>23</sub>N<sub>3</sub>O<sub>5</sub> 349.

## Example 16

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-3-benzylamino-N-benzyl-acrylamide



5        A        solution        of        (3S,        4S) - 3 - (2S-2-  
benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-  
phenoxy-azetidin-2-one (1.1 g, 3.3 mmole) in ethanol (20  
ml) and 5 ml of benzylamine was stirred at room  
temperature overnight. After removal of solvent under  
10      vacuum and lyophilization, the residue was purified by  
silica gel column chromatography using methanol-chloroform  
as eluant. 0.96 g of the title compound was obtained as  
white solid.

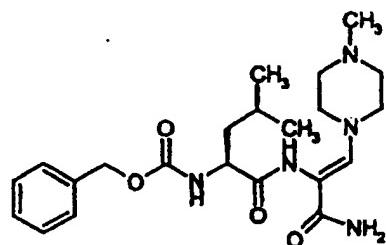
Yield: 55%

15       $^1\text{H-NMR}$ : (DMSO- $\text{d}_6$ ), (ppm): 0.8-1.0 (6H, m), 1.4-1.8  
(3H, m), 3.9-4.1 (1H, m), 4.2-4.4 (4H, m), 4.7-4.9 (2H,  
m), 6.0-6.2 (0.5H, m), 6.6-6.8 (1H, m), 7.1-7.4 (16H,  
m), 7.6-7.8 (1H, m), 8.3-8.5 (0.5H, m), 8.68 (0.5H, s),  
8.85 (0.5H, s).

20      MS (ES+): 529 (M+H), calcd for  $\text{C}_{31}\text{H}_{36}\text{N}_4\text{O}_4$  528.

## Example 17

2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-  
acetamido)-3-(4-methylpiperazino)-acrylamide



5 To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (3 ml) and water (1 ml), 4-methylpiperazine (106 mg, 1.06 mmole) was added and stirred at room  
10 temperature overnight. After removal of acetonitrile under vacuum, the residue was dissolved in ethyl acetate and washed with water, brine and dried with Na<sub>2</sub>SO<sub>4</sub>. After removal of solvent, 30 mg of the title compound was obtained as white solid.

15 Yield: 27%

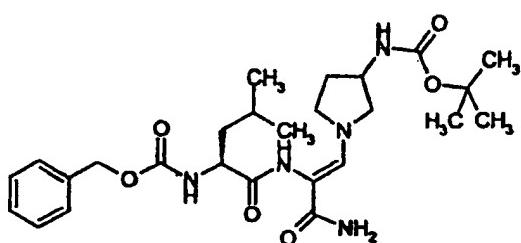
m.p.: 93.5-95 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.0 (6H, m), 1.3-1.8 (3H, m), 2.13 (3H, s), 2.2-2.35 (4H, m), 3.2-3.35 (4H, m), 3.95-4.1 (1H, m), 5.01 (2H, s), 6.29 (2H, s), 7.07 (1H, s), 7.3-7.4 (5H, m), 7.66 (1H, d, J=6.7 Hz), 8.79 (1H, s).

20 MS (ES+): 432 (M+H), calcd for C<sub>22</sub>H<sub>33</sub>N<sub>5</sub>O<sub>4</sub> 431.

## Example 18

- 5 2-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-  
acetamido)-3-(3-tert-butoxycarbonylamino-pyrrolidino)-  
acrylamide



To a solution of (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one (100 mg, 0.257 mmole) in acetonitrile (3 ml) and water (1 ml), 3-tert-butoxycarbonylamino-pyrrolidine (239 mg, 1.28 mmole) was added and stirred at room temperature overnight. After removal of acetonitrile under vacuum, the residue was dissolved in ethyl acetate and washed with water, brine and dried with Na<sub>2</sub>SO<sub>4</sub>. After removal of solvent, 120 mg of the title compound was obtained as white solid.

Yield: 90%

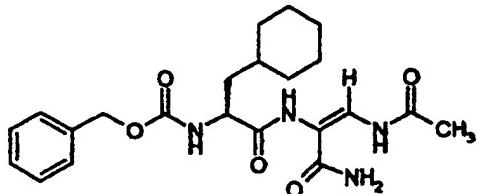
20 m.p.: 140-142 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.0 (6H, m), 1.3-2.0 (5H, m), 1.37 (9H, s), 3.0-3.2 (1H, m), 3.3-3.7 (3H, m), 3.9-4.1 (2H, m), 5.01 (2H, s), 6.25 (2H, s), 7.12 (1H, d, J=6.5 Hz), 7.22 (1H, s), 7.3-7.4 (5H, m), 7.66 (1H, d, J=6.5 Hz), 8.75 (1H, s).

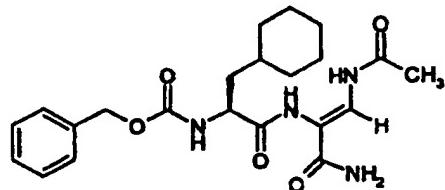
MS (ES+): 518 (M+H), calcd for C<sub>26</sub>H<sub>39</sub>N<sub>5</sub>O<sub>6</sub> 517.

**Example 19a and 19b**

5   (2E)- (2S-2-benzyloxycarbonylamino-2-cyclohexymethyl-  
acetamido) -3-acetamido-acrylamide (19a)



(2Z)- (2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-  
acetamido) -3-acetamido-acrylamide (19b)



10   By a similar method as described in example 10, the title compound was obtained from 2-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-3-amino-acrylamide (example 2).

For (19a):

15   Yield: 21 %

m.p.: 140-142 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.7-1.8 (13H, m), 2.01 (3H, s), 3.95-4.1 (1H, m), 5.02 (2H, m), 6.88 (1H, br), 7.07 (1H, d, J=11 Hz), 7.3-7.4 (5H, m), 7.5 (1H, br), 7.65 (1H, d, J=6 Hz), 9.20 (1H, s), 10.97 (1H, d, J=11 Hz).

20   MS (ES+): (M+H), calcd for C<sub>22</sub>H<sub>30</sub>N<sub>4</sub>O<sub>5</sub> 430.

For (19b) :

Yield: 41 %

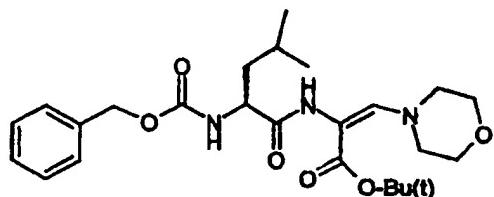
m.p.: 151-153 °C.

<sup>1</sup>H-NMR: (DMSO-d<sub>6</sub>), (ppm): 0.8-1.8 (13H, m), 1.99 (3H, s), 4.0-4.1 (1H, m), 5.04 (2H, m), 7.01 (2H, br), 7.3-7.4 (5H, m), 7.62 (1H, d, J=11.4 Hz), 7.81 (1H, d, J=6.2 Hz), 8.98 (1H, s), 9.11 (1H, d, J=11.4 Hz),  
MS (ES+): (M+H), calcd for C<sub>22</sub>H<sub>30</sub>N<sub>4</sub>O<sub>5</sub> 430.

10

### Example 20

tert-Butyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (20)



15 A mixture of ZLeuGlyOBu<sup>t</sup> (0.5g, 0. mmol), 1-(diethoxymethyl)imidazole (0.36g, mmol) and camphor shulphonic acid (0.056g, mmol) in toluene was treated with morpholine (1.0ml, mmol) and was refluxed for 24hrs. Solvent was removed in vacuo and the crude product  
20 obtained was purified over silica gel column chromatography using a gradient mixture of hexane and ethyl acetate (1:1 to 2:1) gave 20 mg of title compound.

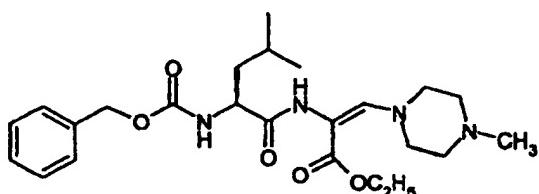
Yield; 3.2%

<sup>1</sup>H NMR (DMSO- d<sub>6</sub>): δ 0.80-0.90(m, 6H), 1.24-1.72(m, 3H),  
25 3.2-3.48(m, 8H), 3.95-4.06(m, 1H), 5.00(AB<sub>q</sub>, 2H, J= 2.7

and 13.0Hz), 7.11(s, 1H), 7.34(s, 5H), 7.43(d, 1H, J= 8.0Hz), 8.49(s, 1H).

### Example 21

5 Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(4-methylpiperazino-1-methylenyl)-acetate  
(21)

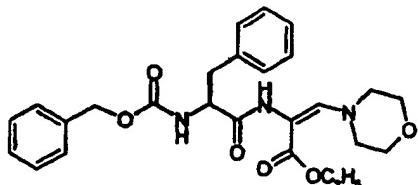


A mixture of ZLeuGlyOEt (0.3g, 0.86mmol), 10 (diethoxymethyl)imidazole (0.28g, 1.27mmol) in toluene was treated with N-methylpiperazine (0.94ml, 8.5mmol) and was refluxed for 24hrs. Solvent was removed in vacuo and the crude product obtained was purified over. Purification of the above crude product over silica gel column chromatography using a mixture of ethyl acetate and methanol (9:1) gave the title compound (0.025g),  
 Yield 6.3%,  
 m. p. 184°C.

15 <sup>1</sup>H NMR (DMSO- d<sub>6</sub>): δ 0.87-0.95(m, 6H), 1.12(t, 3H, J= 7.0Hz), 1.43-1.79(m, 3H), 2.11(s, 3H), 2.21(s, 4H), 3.40(m, 4H), 3.94(q, 2H, J= 7.0Hz), 3.97-4.12(m, 1H), 5.01(AB<sub>q</sub>, 2H, J= 8.3 and 12.6Hz), 7.20(s, 1H), 7.34(s, 5H), 7.44(d, 1H, J= 7.9Hz), 8.53(s, 1H).

## Example 22

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (22)



5 By a similar method as described in example 21, the title compound compound was obtained from Ethyl-2-[2S-(benzyloxycarbonylamino)-2-phenylmethyl-acetamido]-acetate, morpholine and 1-(diethoxymethyl)imidazole.

Yield; 4.5%,

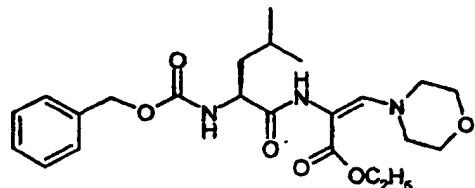
10 m.p. 215-217 °C

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 1.14(t, 3H, J= 7.1Hz), 2.69-3.10(m, 2H), 3.35-3.47(m, 8H), 3.98(q, 2H, J= 6.2Hz), 4.20-4.33(m, 1H), 4.93(AB<sub>q</sub>, 2H, J= 6.7 and 12.7Hz), 7.20-7.40(m, 11H), 7.57(d, 1H, J= 8.4Hz), 8.79(s, 1H).

15

## Example 23

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (23)



20 By a similar method as described in example 21, the title compound compound was obtained from Ethyl-2-[2S-

(benzyloxycarbonylamino)-2-isopropylmethyl-acetamido]-acetate, morpholine and 1-(diethoxymethyl)imidazole

Yield; 16%,

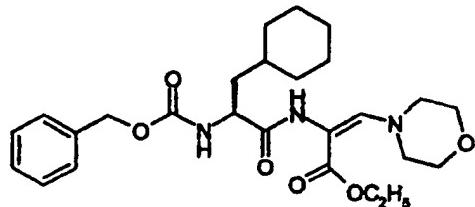
m.p. 139-141 °C

5  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>):  $\delta$  0.87(t, 6H, J= 6.1Hz), 1.13(t, 3H, J= 7.1Hz), 1.43-1.78(m, 3H), 3.38-3.51(m, 8H), 3.90-4.09(m, 3H), 5.00(AB<sub>q</sub>, 2H, J= 2.1 and 12.7Hz), 7.21(s, 1H), 7.34(s, 5H), 7.45(d, 1H, J= 7.5Hz), 8.56(s, 1H).

10

#### Example 24

Ethyl-2-[2S-(benzyloxycarbonylamino)-2-cyclohexylmethyl-acetamido]-2-(morpholino-1-methylenyl)-acetate (24)



15

By a similar method as described in example 21, the title compound compound was obtained from Ethyl-2-[2S-(benzyloxycarbonylamino)-2-cyclohexylmethyl-acetamido]-acetate, morpholine and 1-(diethoxymethyl)imidazole.

20 Yield; 10%,

m.p. 202°C

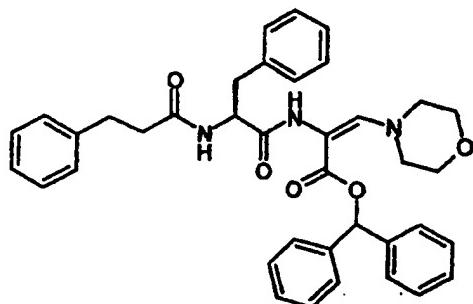
1  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>):  $\delta$  0.75-1.76(m, 16H), 3.35-3.50(m, 8H), 3.90-4.12(m, 3H), 5.00(s, 2H), 7.21(s, 1H), 7.34(s, 5H), 7.45(d, 1H, J= 7.0Hz), 8.54(s, 1H).

25

## Example 25

Diphenylmethyl-2-[2S-(3-phenylpropionylamino)-2-  
phenylmethyl-acetamido]-2-(morpholino-1-methylenyl)-

5 acetate



By a similar method as described in example 21, the title compound compound was obtained from Diphenylmethyl-  
10 2-[2S-(benzyloxycarbonylamino)-2-isopropylmethyli-  
acetamido]-acetate, morpholine and 1-(diethoxymethyl)imidazole.

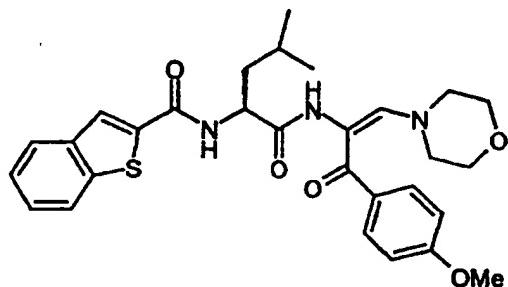
Yield; 27%,

m.p. 167-170 °C

15 <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): 2.32 (t, 2H, J= 8.4Hz), 2.67(t, 2H, J= 7.0Hz), 2.70-3.26(m, 2H), -3.40-3.55(m, 8H), 4.50-4.67(m, 1H), 6.74(s, 1H), 7.10-7.49(m, 20H), 8.27(d, 1H, J= 8.0Hz), 8.95(brs, 1H).

## Example 26

2-[2S-(Benzothiophen-2-carbonylamino)-2-isopropylmethyl-  
acetamido]-2-(morpholino-1-methylenyl)-4'-  
5 methoxyacetophenone (26).



A mixture of N-(benzothiophene-2carbonyl)amino-Leucine (0.332g, 1.14mmol), DCC(0.235g, 1.14mmol) and 1-hydroxy benzotriazole (0.154g, 1.14mmol) in dry THF was stirred under nitrogen at r.t. for 1h and cooled to 0 °C. The suspension obtained was filtered and to the filtrate was added 2-amino-4'-methoxy acetophenone (0.23g, 1.14mmol) followed by triethyl amine (0.127g, 1.25mmol). The reaction mixture was stirred at r.t. for 6 hrs. and evaporated in vacuo to give the crude product. Purification of the above crude product over silica gel column chromatography using a mixture of hexane and ethyl acetate (2:3) gave 288 mg of title compound 2[2S-(benzothiophene-2-carbonyl)amino-2-isopropylmethylacetamido]-(4'methoxy)acetophenone.

Yield; 58%,

<sup>1</sup>H NMR (DMSO- d<sub>6</sub>): δ 0.90-1.00(m, 6H), 1.58-1.82(m, 3H), 3.84(s, 3H), 4.55-4.67(m, 3H), 7.05(d, 2H, J=8.8Hz), 7.44-

7.48(m, 2H), 7.95-8.05(m, 4H), 8.28(s, 1H), 8.35(t, 1H, J=5.9Hz), 8.86(d, 1H, J=8.3Hz).

A mixture of 2-[2S-(benzothiophene-2-carbonyl)-amino-2-isopropylmethyl-acetamido]-4'methoxyacetophenone

5 (0.271g, 0.618mmol) and 1-(diethoxymethyl)imidazole (0.202g, 0.927mmol) in toluene was treated morpholine (0.269g, 3.09mmol) and refluxed at 130 °C over 22 hrs. Toluene was removed in vacuo and the crude product was purified over silica gel column chromatography using a  
10 mixture of ethyl acetate and methanol (9:1) to give 200 mg of title compound.

Yield : 60%.

m.p. 134-136 °C

<sup>1</sup>H NMR (DMSO- d<sub>6</sub>): δ0.82-0.93(m, 6H), 1.15-1.87(m, 3H),  
15 3.40-3.56(m, 8H), 3.75(s, 3H), 4.37-4.50(m, 1H), 6.90(d, 2H, J=8.6Hz), 7.09(s, 1H), 7.41-7.47(m, 4H), 7.95-8.05(m, 2H), 8.25(s, 1H), 8.81(d, 1H, J=7.7Hz), 8.98(s, 1H).

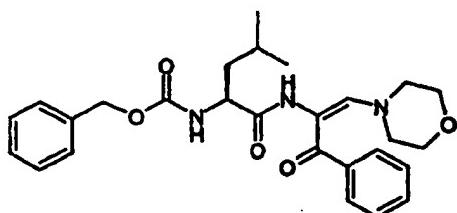
20

25

30

## Example 27

2-[2S-(Benzylloxycarbonylamino)-2-isopropylmethyl-acetamido]-2-(morpholino-1-methyl nyl)-acetophenone (27).



5 By following the procedure as described in example 25, the title compound was obtained from 2-[2S-(Benzylloxycarbonylamino)-2-isopropylmethyl-acetamido]-acetophenone, morpholine and 1-(diethoxymethyl)imidazole.

Yield; 53%,

10 m.p. 107-109 °C

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 0.78-0.85 (m, 6H), 1.06-1.63 (m, 3H), 3.40-3.55 (m, 8H), 3.90-4.05 (m, 1H), 5.00 (ABq, 2H, J= 2.0 and 10.7Hz), 7.13 (s, 1H), 7.35 (s, 5H), 7.38 (s, 5H), 7.44 (d, 1H, J= 8.0Hz), 8.81 (s, 1H).

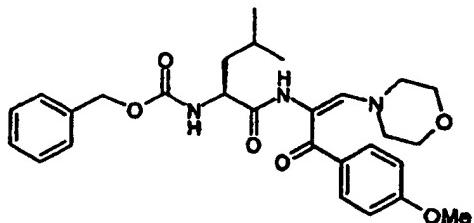
15

20

25

## Example 28

2-[2S-(Benzylloxycarbonylamino)-2-isopropylmethyl-  
acetamido]- 2-(morpholino-1-methylenyl)-4'-  
methoxyacetophenone (28).



5

By following the procedure as described in example 25,  
 the title compound was obtained from 2-[2S-(Benzylloxycarbonylamino)-2-isopropylmethyl-acetamido]- 4'-  
 10 methoxyacetophenone, morpholine and 1-(diethoxymethyl)imidazole.

Yield; 53%,

m.p. 140-143 °C

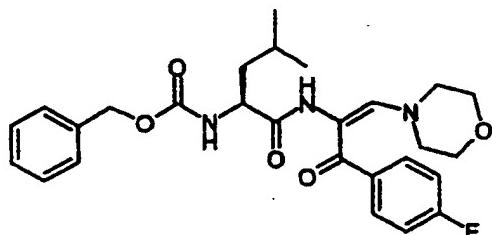
<sup>1</sup>H NMR (DMSO-d<sub>6</sub>) : δ 0.77-0.85 (m, 6H), 1.17-1.63 (m, 3H),  
 15 3.40-3.53 (m, 8H), 3.75 (s, 3H), 3.90-4.05 (m, 1H), 4.99 (s  
 2H), 6.89 (d, 2H, J= 8.6Hz), 7.07 (s, 1H), 7.33-7.45 (s, 8H),  
 8.80 (s, 1H).

20

25

## Example 29

2-[2S-(Benzylloxycarbonylamino)-2-isopropylmethyl-  
acetamido]-  
2-(morpholino-1-methylenyl)-4'-  
fluoroacetophenone (29).



5

By following the procedure as described in example 25, the title compound was obtained from 2-[2S-(Benzylloxycarbonylamino)-2-isopropylmethyl-acetamido]-4'-fluoroacetophenone, morpholine and 1-(diethoxymethyl)imidazole.

Yield; 30%,

m.p. 156-157 °C

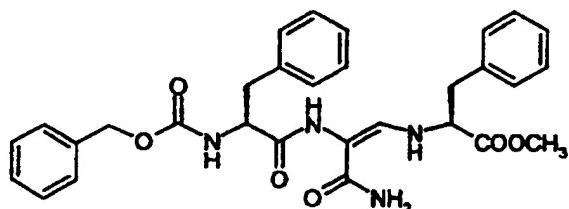
<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 0.80-0.90 (m, 6H), 1.05-1.64 (m, 3H), 3.50 (m, 8H), 3.87-4.03 (m, 1H), 5.00 (s, 2H), 7.15-7.50 (m, 11H), 8.85 (s, 1H).

20

25

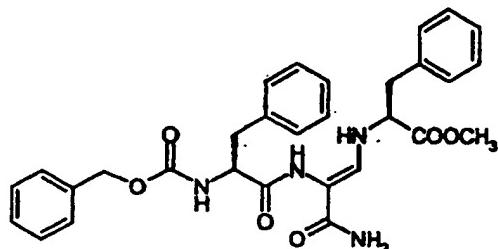
## Example 30a and 30b

(2E) - (2S-2-benzyloxycarbonylamino-2-phenylmethyl-  
acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamid  
(30a)



5

(2Z) - (2S-2-benzyloxycarbonylamino-2-phenylmethyl-  
acetamido)-3-(1-carbomethoxy-2-phenethylamino)-acrylamide  
(30b)



10

By following the procedure as described in example 18, the title compounds were obtained from (3S, 4S)-3-(2S-2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one and methyl ester of phenylalanine.

15 For 30a:

Yield; 34%,

m.p. 71-73 °C

NMR (DMSO-d<sub>6</sub>): 2.75-3.03 (m, 4H), 3.63 (s, 3H), 4.12-4.38 (m, 2H), 4.96 (s, 2H), 6.00 and 6.55 (2br. s, 2H), 6.34 (d, 1H,

$J = 12.2\text{Hz}$ ), 7.20-7.46(m, 15H), 7.67(d, 1H,  $J = 6.0\text{Hz}$ ), 8.30-8.40(m, 1H), 8.70(s, 1H).

For 30b:

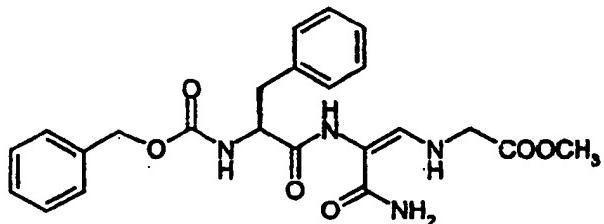
5 Yield; 42%, m.pt. 85-87 °C

NMR(DMSO-d<sub>6</sub>): 2.79-3.15(m, 4H), 3.60(s, 3H), 4.16-4.35(m, 2H), 4.96(AB<sub>q</sub>, 2H,  $J = 2.2$  and 12.6Hz), 5.42-5.53(m, 1H), 6.15(brs, 2H), 7.08(d, 1H,  $J = 13.0\text{Hz}$ ), 7.20-7.30(m, 15H), 7.79(d, 1H,  $J = 6.5\text{Hz}$ ), 8.71(s, 1H).

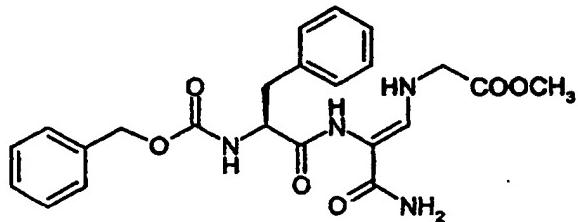
10

Example 31a and 31b

(2E) - (2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-methylamino)-acrylamide (31a)



15 (2E) - (2S-2-benzyloxycarbonylamino-2-phenylmethyl-acetamido)-3-(1-carbomethoxy-methylamino)-acrylamide (31b)



By following the procedure as described in example 18, the title compounds were obtained from (3S, 4S)-3-(2S-

2-benzyloxycarbonylamino-2-isopropylmethyl-acetamido)-4-acetoxy-azetidin-2-one and methyl ester of glycine

For 31a:

Yield; 36%,

5 m.p. 108-110 °C

NMR(DMSO-d<sub>6</sub>): 1.21(t, 3H, J= 7.0Hz), 2.76-3.04(m, 2H), 3.94(d, 2H, J= 6.2Hz), 4.11(q, 2H, J= 7.0Hz), 4.11-4.25(m, 1H), 4.96(s, 2H), 6.02 and 6.53(2brs, 2H), 6.30(d, 1H, J= 12.5Hz), 7.26-7.35(m, 10H), 7.67(d, 1H, J= 6.9Hz), 8.12-10 8.25(m, 1H), 8.72(s, 1H).

For 31b:

Yield; 41%,

m.p. 145-147 °C

15 NMR(DMSO-d<sub>6</sub>): 1.20(t, 3H, J= 7.4Hz), 2.75-3.15(m, 2H), 3.88(d, 2H, J= 5.9Hz), 4.16(q, 2H, J= 7.4Hz), 4.25-4.87(m, 1H), 4.98(s, 2H), 5.22-5.35(m, 1H), 6.20(brs, 1H), 7.05(d, 1H, J= 11.8Hz), 7.19-7.35(m, 10H), 7.75(d, 1H, J= 6.6Hz), 8.70(s, 1H).

20

#### Biological Example

Testing of inhibitors for inhibition of cathepsin B, L, K and S.

25

#### In vitro assay procedure for cathepsin B

The compounds of formula I were tested for inhibition of cathepsin B using the known method (A.J. Barret et al., Biochem. J. 1982, 201, 189-198). To a 170 µl of enzyme-buffer mixture (enzyme: r rat cathepsin B, diluted to give approximate 10 F units/min, buffer: 56 mM sodium

acetate, 1.124 mM EDTA, 10 mM DTT, pH 5.1) a 10  $\mu$ L of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20  $\mu$ l of 5 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to 5 initiate reaction. Reading is followed up for 10 min at the fluoroscan reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC<sub>50</sub> is determined using a 10 linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin L

To a 170  $\mu$ l of enzyme-buffer mixture (enzyme: r rat 15 cathepsin L, diluted to give approximate 15 F units/min, buffer: 58.8 mM sodium citrate, 1.18 mM EDTA, 235 mM sodium chloride, 5 mM DTT, pH 5.0) a 10  $\mu$ L of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20  $\mu$ l of 1 mM substrate (N-CBZ-Phe-20 Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor 25 concentration is obtained, and IC<sub>50</sub> is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin K

30 To a 170  $\mu$ l of enzyme-buffer mixture (enzyme: r cathepsin K, diluted to give approximate 30 F units/min,

buffer: 100 mM sodium acetate, 5 mM EDTA, 20 mM L-cysteine, 0.01% Brij, pH 5.5) a 10 µL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 µL of 2.7 mM substrate (N-CBZ-5 Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan II plate reader (excitation at 380 nm emission at 460 nm).

10 A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC<sub>50</sub> is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

In vitro assay procedure for cathepsin S

15 To a 170 µl of enzyme-buffer mixture (enzyme: r cathepsin S, diluted to give approximate 30 F units/min, buffer: 100 mM sodium phosphate, 1 mM EDTA, 5 mM DTT, 0.01% Brij, pH 6.5) a 10 µL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room 20 temperature, a 20 µL of 12 mM substrate (N-Cbz-Val-Val-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan II plate reader (excitation at 380 nm emission at 460 nm).

25 A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC<sub>50</sub> is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

Table 1. In vitro inhibitory activity of compounds on cysteine proteases

Example No. IC<sub>50</sub> (μM)

5

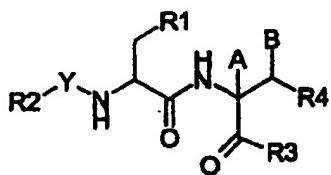
		Cathepsin B	Cathepsin L	Cathepsin K	Cathepsin S
	1	4.35	0.094	0.011	0.069
10	2	1.17	0.072	1.78	0.026
	3	1.42	0.0055	0.23	0.26
	4	2.63	0.015	1.2	0.0087
	5	2.28	0.064	0.0031	0.061
	6	0.37	0.075	0.003	0.05
15	7	1.96	0.23	0.012	0.18
	8	37.1	2.36	0.33	6.9
	9	0.89	0.062	0.014	0.05
	10a	45.21	8.62	1.42	0.013
	10b	50.51	>64	10.73	>3.2
20	11	2.4	0.11	0.014	0.015
	12	1.64	0.076	2.06	0.0035
	13	1.4	0.004	0.4	0.004
	14	0.98	0.004	1.13	0.004
	15	9.5	0.17	0.018	0.075
25	16	0.15	0.015	0.01	0.026
	17	1.9	0.23	0.019	0.18
	18	1.9	0.12	0.0096	0.039

30

	19a	>58	>58	>58	1.86
	19b	>58	58	>58	5.23
	20	0.42	0.08	0.004	0.38
	21	8.21	0.21	0.087	0.3
5	22	0.08	0.04	0.04	0.42
	23	0.45	0.089	0.011	0.038
	24	0.082	0.082	0.37	0.0033
	25	0.06	0.18	0.065	0.18
	26	1.47	0.075	0.015	0.99
	27	32.27	4.24	0.14	52.2
10	28	1.96	0.27	0.016	1.3
	29	50.3	2.01	0.34	50.3
	30a	1.83	0.048	0.28	0.57
	30b	1.83	0.035	0.28	1.21
	31a	0.66	0.017	0.13	0.7
15	<u>31b</u>	<u>0.43</u>	<u>0.017</u>	<u>0.09</u>	<u>0.43</u>

## CLAIMS

## 1. A compound of formula (I)



5

(I)

wherein:

- Y represents -C(O)- or -S(O<sub>2</sub>)-;
- 10 R<sub>1</sub> represents a radical of formula R<sub>6</sub>- (ALK)<sub>p</sub>- (Z)<sub>n</sub>- (ALK)<sub>q</sub>-  
wherein Z represents -O- or -S-, ALK represents a  
divalent C<sub>1</sub>-C<sub>3</sub>alkyl or halogen-substituted C<sub>1</sub>-C<sub>3</sub>alkyl  
radical, p and q are independently 0 or 1, n is 0 or 1  
when q is 1 and n is 0 when q is 0, and R<sub>6</sub> is hydrogen or  
15 an optionally substituted C<sub>1</sub>-C<sub>6</sub>alkyl, C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-  
C<sub>6</sub>alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic  
group; or R<sub>1</sub> together with the carbon atom to which it is  
attached forms a cycloalkyl ring;
- 20 R<sub>2</sub> represents -OR<sub>5</sub> or -R<sub>5</sub>;
- R<sub>5</sub> represents a radical of formula R<sub>7</sub>- (A)<sub>t</sub>- wherein t is 0  
or 1; A represents (i) an optionally substituted divalent  
C<sub>1</sub>-C<sub>6</sub>alkyl, radical which may be interrupted by one or  
25 more non-adjacent -O-, -S- or -NH- linkages, or (ii) a  
divalent C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-C<sub>6</sub>alkynyl, cycloalkyl,

cycloalkenyl, aryl or heterocyclic radical, or (iii) a -NH- link; and R<sub>7</sub> represents hydrogen or an optionally substituted C<sub>1</sub>-C<sub>6</sub>alkyl, C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-C<sub>6</sub>alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group;

5

R<sub>3</sub> represents (I) an optionally substituted C<sub>1</sub>-C<sub>6</sub>alkyl, C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-C<sub>6</sub>alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclic group or (ii) NHR<sub>8</sub> or N(R<sub>8</sub>)<sub>2</sub> or (iii) OR<sub>8</sub> wherein R<sub>8</sub> represents hydrogen or an optionally substituted C<sub>1</sub>-C<sub>6</sub>alkyl, C<sub>2</sub>-C<sub>6</sub>alkenyl, C<sub>2</sub>-C<sub>6</sub>alkynyl, cycloalkyl, cycloalkenyl or aryl;

A and B taken together represent a bond and R<sub>4</sub> represents a hydroxy or substituted hydroxy group or an amino or primary or secondary amino group, or A represents hydrogen and B and R<sub>4</sub> each independently represents a hydroxy or substituted hydroxy group;

or a pharmaceutically acceptable salt, hydrate or solvate thereof.

2. A compound as claimed in claim 1 wherein Y is -C(O)-.

3. A compound as claimed in claim 1 or claim 2 wherein R<sub>1</sub> is a phenyl group which may be substituted by one or more of hydroxy, halogen, methoxy, methyl, isopropyl, tert-butyl and trifluoromethyl; isopropyl, cyclohexyl; 3-pyridinyl; naphthyl; biphenyl; 2-thienyl; 3,4-methylenedioxyphenyl; 3,4-ethylenedioxy-phenyl; benzothienyl; thiazolyl; quinolinyl; isoquinolinyl;

tetrahydroquinolinyl; tetrahydronaphthyl;  
aminonaphthyl; or acetamidonaphthyl.

4. A compound as claimed in claim 1 or claim 2 wherein R<sub>1</sub>  
5 phenyl, isopropyl, cyclohexyl or 3-pyridinyl.

5. A compound as claimed in any of the preceding claims  
wherein R<sub>2</sub> is benzyloxy, 3-phenylpropyloxy, 3-  
phenylpropyl, 3-phenylprop-1-enyl, 6-N,N-  
10 dibenzyloxycarbonylguanidino-hexyl, 6-guanidino-hexyl,  
methoxy-methyleneoxy-methyl, 2-amino-ethoxy-methyl, 3-  
(pyridin-3- or 4-yl)-propyl, or 3-(pyridin-3- or 4-yl)-  
prop-1-enyl.

15 6. A compound as claimed in any of claims 1 to 4 wherein R<sub>3</sub>  
may be, for example, methyl, ethyl, isopropyl, t-butyl,  
cyclohexyl, phenyl, 4-methoxyphenyl, 4-fluorophenyl,  
pyridyl, -NH<sub>2</sub>, methylamino, dimethylamino, benzylamino,  
piperidino, morpholino, piperazino, N-methylpiperazino,  
20 methoxy, ethoxy, t-butyloxy or phenoxy.

7. A compound as claimed in any of the preceding claims  
wherein A and B taken together represent a bond, and R<sub>4</sub>  
is -NH<sub>2</sub>, acetylarnino, methylarnino, dimethylarnino,  
25 benzylarnino, morpholinyl, piperidino, morpholino,  
piperazino or N-methylpiperazino, (methoxycarbonyl)-  
methylarnino, (methoxycarbonyl)-phenethylarnino, -OH,  
methoxy, allyloxy, benzyloxy.

8. A compound as claimed in any of claims 1 to 6 wherein A represents hydrogen and B and R<sub>4</sub> each independently represents a hydroxygroup.

5 9. A compound as claimed in claim 1 which is specifically named and characterised in any of the Examples herein.

10. A pharmaceutical composition containing a compound as claimed in any of the preceding claims and a pharmaceutically acceptable carrier.

11. The use of a compound as claimed in any of claims 1 to 9 in the preparation of a composition for inhibiting cysteine protease activity in the body of a mammal suffering a disease mediated by such activity.

12. A method of treatment of an animal suffering from a disease mediated by cysteine protease activity, which method comprises administering to the mammal a sufficient amount of a compound as claimed in any of claims 1 to 9 to inhibit such activity.

13. The use as claimed in claim 11 or a method as claimed in claim 12 wherein the disease is muscular dystrophy, osteoporosis, tumour metastasis, rheumatoid arthritis, neuronal or cardiac ischaemia, allergic immune response, or protozoal or bacterial disease.

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number  
**WO 01/09169 A3**(51) International Patent Classification<sup>7</sup>: C07K 5/062, 5/065, C07D 295/12, A61K 38/05, 31/5375, 31/381, A61P 19/00, 31/00, 35/00, 37/00, 9/00, 25/00

(CA). MICETICH, Ronald, George [CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA). WHITTAKER, Mark [GB/GB]; British Biotech Pharmaceuticals Ltd., Watlington Road, Cowley, Oxford OX4 5LY (GB).

(21) International Application Number: PCT/GB00/02830

(22) International Filing Date: 21 July 2000 (21.07.2000)

(74) Agents: WALSH, David, Patrick et al.; Appleyard Lees, 15 Clare Road, Halifax HX1 2HY (GB).

(25) Filing Language: English

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(26) Publication Language: English

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).(30) Priority Data:  
9917909.5 31 July 1999 (31.07.1999) GB(71) Applicant (*for all designated States except US*): NAEJA PHARMACEUTICAL INC [CA/CA]; #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA).**Published:**

(72) Inventors; and

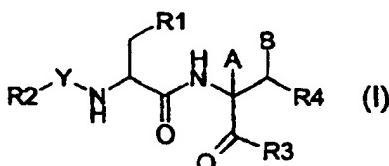
— with international search report

(75) Inventors/Applicants (*for US only*): SINGH, Rajeshwar [CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA). ZHOU, Nan [CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA). REDDY, Andhe, V., N. [CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA). THOMAS, George [CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA). DING, Qizhu [CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2 (CA). KALETA, Jadwiga [CA/CA]; Naeja Pharmaceutical Inc, #2, 4290 - 91A Street, Edmonton, Alberta T6E 5V2(88) Date of publication of the international search report:  
13 September 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/09169 A3

(54) Title: CYSTEINE PROTEASE INHIBITORS



(57) Abstract: This invention relates to derivatives of alpha-amino acid amides, to pharmaceutical compositions containing such compounds, and to their use in medicine as inhibitors of cysteine proteases, particularly the cathepsins. A compound of formula (I) is described or a pharmaceutically acceptable salt, hydrate or solvate thereof. Pharmaceutically acceptable salts of the compounds of this invention include the sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid salts.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 00/02830

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 7	C07K5/062	C07K5/065	C07D295/12	A61K38/05	A61K31/5375
	A61K31/381	A61P19/00	A61P31/00	A61P35/00	A61P37/00
	A61P9/00	A61P25/00			

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DORAN, JOHN D. ET AL.: "DEACYLATION AND REACYLATION FOR A SERIES OF ACYL CYSTEINE PROTEASES INCLUDING ACYL GROUPS DERIVED FROM NOVEL CHROMOPHORIC SUBSTRATES" BIOCHEMISTRY (1996) 35(38) 12487-12494, XP002163740	
A	WO 96 32408 A (SYNPHAR LAB INC) 17 October 1996 (1996-10-17) cited in the application	
A	WO 98 12176 A (SYNPHAR LAB INC) 26 March 1998 (1998-03-26) cited in the application	
	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

### \* Special categories of cited documents:

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- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the International search

23 March 2001

Date of mailing of the International search report

05/04/2001

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/02830

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of documents, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BAGGIO RICKY ET AL: "From poor substrates to good inhibitors: Design of inhibitors for serine and thiol proteases." BIOCHEMISTRY, vol. 35, no. 11, 1996, pages 3351-3353, XP002163741 ISSN: 0006-2960	
A	DUFFY K J ET AL: "Design and synthesis of diaminopyrrolidinone inhibitors of human osteoclast cathepsin K" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, GB, OXFORD, vol. 9, no. 14, 19 July 1999 (1999-07-19), pages 1907-1910, XP004171607 ISSN: 0960-894X	

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/02830

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9812176	A 26-03-1998	AU 4133697 A EP 0950046 A US 5959123 A	14-04-1998 20-10-1999 28-09-1999